

Biomarkers in neurocritical care

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INTRODUCTION

One of the great challenges faced by practitioners is reaching the correct diagnosis in patients with acute neurological problems. Usually, excellent history taking complemented with skilful neurological examinations will shed light on the possible diagnosis. However, in practice, the inconspicuousness of disease often eclipses the real problem, and the lack of readily reliable data may delay or misdirect the care.

Given these circumstances, biomarkers play a role and act as a surrogate measure. In a highly dynamic Neurointensive Care Unit (NICU) environment, it can help guide the treatment decisions or provide risk stratification. It is not meant to replace the traditional methods, but should rather serve as a useful adjunctive tool. We aim to review the different types, strengths and limitation of biomarkers in this setting.

DEFINITION OF BIOMARKERS

According to the Biomarkers Definitions Working Group paper, a biomarker is a characteristic, that is, objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention.^[1] There are several features that are desirable for biomarkers to be useful in the Neurocritical Care Unit (NICU). They have to be brain specific; they have to increase or decrease significantly during the relevant neurological insult; they must be available within a few hours. All these features will help a treating physician to make better diagnosis and direct care.

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HISTORY OF BIOMARKERS

Since the 1950s, interests in biomarkers of neuronal injuries have increased significantly. Most of the earliest work involves studies in traumatic brain injury (TBI) and cerebral ischaemia. In 1976, Rudman *et al.* published a classic study on cerebrospinal fluid (CSF) cyclic adenosine monophosphate levels in TBI as a putative marker of the depth of coma after injury.^[2,3] In 1988, Vaagenes *et al.* studied the levels of brain creatine kinase (CK) in CSF in dogs after cardiac arrest and shows that peak activity at 48–72 h post-arrest correlated with poor outcome.^[4] This was translated to human 6 years later and found that the increase in CK activity in CSF reflects permanent brain damage.^[5] Despite the ubiquitous use of CK to investigate cardiac injury, it had never been part of the routine blood work for neurological injury.

For the next 25 years, the list of putative neurological biomarkers continued to grow as did the conflicting results of their utility in different types of brain injury. The ones that have received the most attention are S-100B and neuron-specific enolase (NSE). Both were first described in the 1960s. These biomarkers have shown effectiveness as a diagnostic and prognostic marker in the setting of wide range acute neurological diseases.

More recently, a systematic review on biomarkers recommended against the routine use of biomarkers for outcome prognostication for all disorders except hypoxic ischemic encephalopathic injuries, where NSE may be used in conjunction with clinical data for neurologic prognostication.^[6]

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APPLICATIONS IN THE NEUROINTENSIVE CARE UNIT

The majority of the patients in the NICU suffer from acute ischemic stroke (AIS), TBI, intracerebral haemorrhage (ICH) or sub-arachnoid haemorrhage (SAH). All these involve an active process going on with the nervous tissues. The outcomes of these patients often depend on how well we can mitigate the disastrous pathophysiology. Given the nature of most of the neurocritical diseases, a good neurological examination may be difficult to obtain, and the procurement of imaging studies may also be delayed for various reasons. These factors lead to the search for neuro-biomarkers. Public interest involving TBI in sports and accidents, along with the military personnel from the Iraq and Afghanistan Wars have also helped to fuel the development of biomarkers with both public and private funding.

Of the biomarkers that have been studied, NSE, S-100B, glial fibrillary acidic protein (GFAP) and ubiquitin C-terminal hydrolase L1 (UCH-L1) are the most well researched [Tables 1 and 2]. Newer biomarkers such as neurofilaments, amyloid beta, spectrin, apolipoprotein E (APOE) and many others are currently under investigation.

APPROACHES

Traditionally, researchers have focused on the investigation of a single or few molecules processes related specifically to the pathological process in the brain, such as the use of S-100B and NSE. In this review, we will discuss some of the newer approaches and what lies in the future [Figure 1].

Proteomic approach

Acute neurological diseases set into motion a complex secondary injury cascade. Utilising the proteomic approaches allow assessment of hundreds or thousands of proteins simultaneously. Several traditional techniques have been used including gel electrophoresis, mass spectrometry, antibody arrays and high-throughput immunoblotting, which are then combined with bioinformatics.^[7] For example, Jenkins *et al.* used two-dimensional gel electrophoresis in TBI and identified over 1500 proteins with 10% demonstrating a 10-fold change between injury and control conditions.^[8] Recently, serum amyloid A using the same method was identified in children with mild TBI.^[9] Overall, proteomic screenings of blood and CSF of acute neurological injuries hold promise in the identification of newer biomarkers.

Lipidomics approach

Lipid peroxidation has been long associated with brain injury.^[10] Lipidomics, first introduced by Han and Gross in 2003, is the qualitative and quantitative analyses of the lipid components from serum, plasma, tissue or

cell.^[11] Cardiolipin, a phospholipid, found exclusively in the mitochondria. Cytochrome C in the mitochondria interacts with cardiolipin and acts as a cardiolipin oxygenase. This oxygenase is activated in a variety of cellular stresses which subsequently trigger apoptotic cell death.^[12,13] Limiting factors to this approach include the origin of the lipids,^[14] and the lack of proper oxidised phospholipid standards.^[13]

Blood-based genetic marker

Several studies have shown that APOE genotype may be related to clinical outcome in brain injuries. Athletes with APOE $\epsilon 4$ reported greater symptomatology post-concussion than those without.^[15] It is also associated with an increased risk of unfavourable outcome in TBI^[16] and SAH.^[17] There is also strong associations between APOE variants and lobar ICH.^[18] Suggesting that low-density lipoprotein levels may modulate this effect.^[19] Possible limitations of this genetic marker include the fact that it may not reflect the current brain injury pathology; it may be as a result of systemic lipid metabolism, innate immunity or endocytic trafficking that act as contributors to brain injuries, as suggested in the pathology of Alzheimer's disease.^[20] Despite not being conclusive, it holds a promising future.

MicroRNAs

MicroRNAs (miRNAs) was first identified in 1993^[21] are an abundant class of short, non-coding RNA molecules, approximately 22 nucleotides (nt) in length, which regulate gene expression through RNA interference.^[22] It has been linked with neuroinflammation in acute brain injury, infection, neuroimmune and neurodegenerative diseases.^[23] Exposure to any pathogen leads to significant changes in the expression of specific miRNAs in immune cells, such as in the case of miR-155 and miR-146a, which have an important role in the Japanese encephalitis.^[23,24] Several circulating miRNAs have been shown to change in the plasma of TBI patients.^[25] Bioinformatic analysis indicates that miR-let-7i may regulate TBI-related proteins and inflammatory cytokines, including S-100B, GFAP and UCH-L1, suggesting the potential use of it as diagnostic biomarkers.^[26] Ischaemic stroke patients also display changes in the level of it.^[27] Although still in its early stages research, it holds great potentials to monitor the courses of disease.

Other approaches

The search for pathological fingerprint continues with excitement in the era of advancement in translational medicine and unfolds the possibilities of newer approaches. Multiplex bead technology provides quantitative measurement of large numbers of analytes and this will broaden attempts at discovering newer biomarkers.^[28] Exosomes are nanovesicles secreted into the blood upon internal vesicle fusion with the

Table 1: A summary of potentially useful biomarkers in the Neurocritical Care Units

Marker	Structure affected	Findings in relation to brain injury	
		CSF	Blood
S-100B	Astroglial cells	Elevated	Elevated but may be confounded by extracerebral tissues
GFAP	Astroglial cells	Elevated	Elevated
MBP	Axon	N/A	Not sensitive
NFLP	Axon	Peak at 4-10 days post-injury	N/A
NSE	Neuron	Elevated, but may be confounded by RBC lysis	
UCH-L1	Neuron	Elevated	Elevated
Amyloid beta 40, 42	Plaque pathology	No change	N/A
MMP-9	Blood-brain barrier	Elevated	Elevated
SBDP 145, 150	Membranes, cytoskeleton	Elevated	Elevated
Total tau protein	Axon	Peak at 4-10 days post-injury	Elevated

Other potential biomarkers: Leptin; glutamate; interleukins; APOE; copeptin; BDNF

NSE = Neuron-specific enolase, MBP = Myelin basic protein, GFAP = Glial fibrillary acidic protein, UCH-L1 = Ubiquitin C-terminal hydrolase, NFLP = Neurofilament light polypeptide, MMP = Matrix metalloproteinases, BDNF = Brain-derived neurotrophic factor, SBDP = Spectrin breakdown product, N/A = Not available, CSF = Cerebrospinal fluid, RBC = Red blood cell, APOE = Apolipoprotein E

Table 2: A summary of the types of tests in the Neurocritical Care Units

Diseases	Type of tests	Potential biomarkers
Intracranial hypertension	Head CT, MRI brain, ultrasound, ICP monitor	IL-6
Ischaemic stroke	Head CT, MRI DWI-ADC and angiography	S-100B
Intracerebral haemorrhage	Head CT, MRI SWI FFE, angiography	Leptin in basal ganglia haemorrhage, fibrinogen in post-tPA
Traumatic brain injury	Head CT, MRI brain, MRS and DTI	S-100B, NSE, UCH-L1, NFLP, MBP, GFAP
SAH vasospasm	TCD, CT angiography, invasive cerebral angiography and EEG	Nitrate, nitrite, ADMA, S-100B
Status epilepticus	EEG, MRI	UCH-L1, MiRNA
Cardiac injury	EKG, 2D/3D echogram and MRI heart	Troponin, CKMB

CT = Computed tomography, MRI = Magnetic resonance imaging, ICP = Intracranial pressure, DWI = Diffusion-weighted imaging, ADC = Apparent diffusion coefficient, MRS = Magnetic resonance spectroscopy, DTI = Diffusion tensor imaging, TCD = Transcranial Doppler ultrasound, EEG = Electroencephalogram, EKG = Electrocardiogram, CK = Creatine kinase, NSE = Neuron-specific enolase, MBP = Myelin basic protein, GFAP = Glial fibrillary acidic protein, UCH-L1 = Ubiquitin C-terminal hydrolase, NFLP = Neurofilament light polypeptide, MMP = Matrix metalloproteinases, ADMA = Asymmetric dimethylarginine, IL-6 = Interleukin-6, 2D = Two-dimensional, 3D = Three-dimensional, MiRNA = MicroRNA, SWI = Susceptibility-weighted imaging, FFE = Fast field echo, SAH = Sub-arachnoid haemorrhage

plasma membrane and has been implicated in the pathophysiology of neuronal injuries. It has already

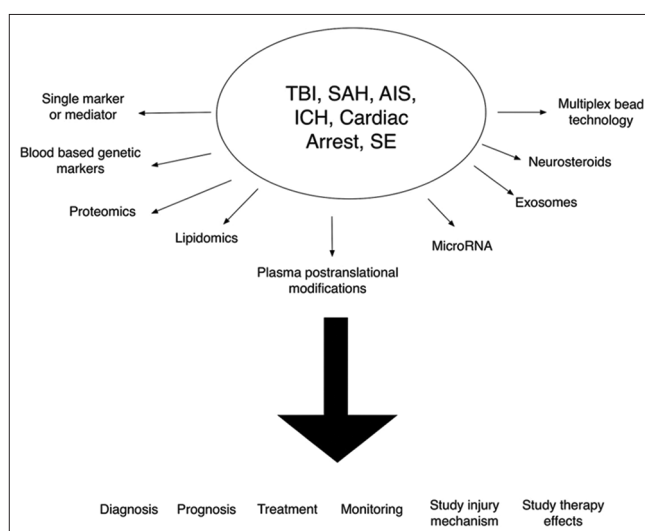


Figure 1: Biomarkers approaches in acute neurological diseases. TBI = Traumatic brain injury, SAH = Sub-arachnoid haemorrhage, AIS = Acute ischaemic stroke, ICH = Intracerebral haemorrhage, SE = Status epilepticus

been used to look for biomarkers in Alzheimer's disease and also being proposed as a possible treatment of neurological injuries.^[29] Plasma post-translational modifications are non-DNA-coded modifications to the structure of proteins that generate novel and unique parts of a protein is another interesting aspect, for example, phosphorylated tau in Alzheimer's disease.^[30] Last, neurosteroids are endogenous brain molecules, and many demonstrate pleiotropic actions that are potentially relevant to TBI and its therapeutics.^[31] Although much work is on-going, these promising discoveries could yield transformative approaches in the diagnosis of acute neurological diseases.

WHAT DO WE KNOW SO FAR REGARDING SPECIFIC BIOMARKERS?

S-100B

First described in 1965,^[32] it is found in the cytosol of central nervous system (CNS) glial cells predominantly the astrocytes, but also extracranially, such as chondrocytes, melanocytes and adipocytes.^[33] It is the first identified member of the S-100 protein multigenic family and participates in an extra- and intra-cellular regulation of a cellular calcium metabolism.^[34,35] It can be detected in blood and CSF.

A few studies showed that S-100B is elevated in SAH compared to healthy subjects and is associated with vasospasm and poor outcome.^[36-38] External ventricular drain in SAH is associated with decreased blood S-100B, which may confound the usability of it.^[38]

A major limitation of S-100B is the acceptability range. At present, none of the reviewed assays established an acceptable range.^[39] The other drawback is its short half-life of 2 h,^[40] therefore, making it only relatively useful in the most severe form of brain injuries.^[41]

Neuron-specific enolase

NSE is a glycolytic enzyme found predominantly in the neuronal cytoplasm. First described in the 1960s,^[32] and has shown sensitivity or specificity in TBI, stroke and cardiac arrest and does not appear to exhibit age-dependent liabilities as seen in S-100B.^[3] Studies found that patients with NSE >28–97 µg/L post-cardiac arrest had poor outcome.^[6,42-45] Another prospective cohort study on 61 patients treated with therapeutic hypothermia after cardiac arrest showed that NSE and electroencephalogram findings were strongly correlated and while five survivors (3 with good outcome) had NSE levels 33 µg/L,^[46] which raised caution on the validity of NSE in the era of therapeutic hypothermia. However, a recently published randomised study of 686 patients to targeted temperature management at either 33°C or 36°C concluded that serial high NSE values have a high predictive value of poor outcome in cardiac arrest patients.^[47] An interesting study also found that in pre-eclampsia, the levels of NSE remained high throughout pregnancy,^[48] therefore raising the possibility of using it to monitor such a condition. As a result of some of these studies, a weak recommendation on the use of serum NSE in conjunction with clinical data for neurologic prognostication was given.^[6] Last, NSE is not a biomarker to be used alone as it has also been implicated as a marker for neuroendocrine bladder of tumour, small cell lung cancer and neuroblastomas.^[49]

Glial fibrillary acidic protein

GFAP is an intermediate filament protein that is only found in the glial cells of the CNS. First described in

1971,^[50] the usefulness of it as biomarkers has been reported in several neurological injuries. It has been shown to have excellent specificity and moderate sensitivity for TBI, while also having good specificity for computed tomography (CT)-confirmed brain injury^[51] and shown to have higher levels in patients with mass lesions compared with diffuse injury.^[52] These raise the redundancy of this biomarkers, as CT scans are readily available in most hospitals. In a recent prospective cohort study of 67 patients, serum GFAP levels on admission and during the first 5 days of injury were increased in patients with severe TBI and were predictive of neurological outcome at 6 months.^[53] However, they do not add predictive power to commonly used prognostic variables in a TBI population of varying severities.^[54] Overall, GFAP has the potential to be a useful biomarker, but more studies need to be done.

Matrix metalloproteinases

Matrix metalloproteinases (MMP) belong to a large family of proteolytic enzymes that degrades basement membrane components such as collagen IV, laminin and fibronectin, which are the major constituents of the blood-brain barrier.^[55] The presence of some of it such as MMP-2 and MMP-9 has been implicated as a negative prognostic factor in stroke.^[56,57] It is also implicated in other pathogenic mechanisms such as post-tissue plasminogen activator (tPA) haemorrhage in stroke, ICH, SAH and TBI. Higher levels are associated with thrombolysis failure.^[58-61] MMP-9 has also been correlated with haemorrhage transformation^[62] and malignant cerebral oedema^[63,64] in AIS. Plasma levels of cellular fibronectin 3.6 µg/mL and of MMP-9 140 ng/mL have been associated with parenchymal hematoma after treatment with rt-PA in patients with AIS in a multicentre confirmatory study.^[65,66] Therefore, in the last two decades, it is one of the most vigorously studied biomarkers for risk stratification and even as a possible neuroprotection agent through the inhibition of it.^[67] Despite all these, MMPs have not shown sufficient sensitivity and specificity for use in the clinical setting. They also have shown weak discriminative ability between AIS and stroke mimics in comparison to baseline clinical parameters, with non-significant improvement in receiver operating characteristic area under the curve.^[68]

Ubiquitin C-terminal hydrolase L1

UCH-L1 is a protein that is involved in the addition or removal of ubiquitin from proteins that are destined for metabolism. First detected in 1980 as protein gene product 9.5.^[69] It presents in human brain at concentrations at least 50-fold greater than in other organs.^[70] However, its exclusivity was questioned when it was also found in non-neuroendocrine carcinomas, such as those of the breast, kidney, prostate, pancreas, lung and colon.^[71] Increased CSF and blood concentrations have

been associated with neuron destruction and increased blood–brain barrier permeability.^[72] It has also been reported in neurodegenerative diseases.^[73] Remarkably, this neuronal protein can be readily detected in CSF and blood very early after brain injury,^[74] status epilepticus^[75] and carbon monoxide poisoning^[76] which allow it to be used as valuable time-window biomarkers for potential neuroprotective strategies. However, in a recently published prospective study of 324 patients with TBI, despite significantly associated with outcome, it does not add predictive power to commonly used prognostic variables.^[54]

Others

Other biomarkers may also have the potential to either being used alone or being used along with others [Table 1]. For example, fibrinogen level reduction by more than 200 mg/dL after tPA shows a higher risk of symptomatic ICH and, therefore being suggested to monitor post-rt-PA haemorrhage.^[77] However, a multicentre retrospective study published recently concluded that it did not significantly reduce the likelihood of in-hospital mortality or hematoma expansion.^[78] Thereby, raising the futility of fibrinogen testing post-tPA. Inflammatory proteins such as interleukin-6 have also been shown to be elevated in CSF in TBI.^[79] However, given its low specificity, it has not been applied clinically. Serum and CSF increased tumour necrosis factor- α levels have been described in patients following a severe TBI;^[80] however, these increased levels did not equate to an increased mortality rate,^[81] along with its low specificity and sensitivity, limiting its use clinically. Future potential markers include spectrin breakdown proteins, microtubule-associated protein, soluble urokinase plasminogen activator receptor and many other more.

LIMITATIONS

Recent reviews summarised the potentials of some of the most common biomarkers discussed^[6,49] and concluded that no single biomarker alone has been proven to have substantial clinical use and more research needs to be undertaken.

Despite progress over the last 25 years in translational science, much of the work has followed a typical pattern. After an initial period of optimism, there is, then, a realisation that although an individual biomarker may be of some use, there are often multiple confounding factors.^[41]

Unlike most organs in the body, the brain itself consists of multiple sub-structures that may share the same biomarkers that serve very different functions. Many of these sub-structures are small and when damaged, it may have a huge morbidity impact. For instance, a small

injury to the pons and frontal lobe may lead to equal elevation of biomarkers, but the consequences are widely different. Hence, how do we interpret it meaningfully?

The rising costs of medical care also raise the concern of the practicality of these tests. Even when proven to be clinically useful, the cost of it has to be compared with more traditional approaches. Last, there is also a lack of consistency across research methods on these topics, and these discrepancies hamper the progress. Qualification of a predictive biomarker signature must be based on randomised prospective clinical trials to demonstrate predictability in the biomarker-defined responders.^[82]

THE FUTURE

New developments in the field of proteomics, lipidomic, genetic markers, exosomes and miRNAs hold great promise in the discovery of newer makers, with trend in the development of personalised medicine, these approaches may even be used as treatment. Therefore, despite many inconclusive and even conflicting studies, the future of biomarkers look very bright.

CONCLUSIONS

Despite multiple studies and the enthusiasm towards the development of it, no single biomarker has proven to be applicable clinically. In the foreseeable future, it may be used as an adjunct, supplementing a good neurological examination and neuroimaging to help in the diagnosis and prognostication; this incorporation will be an important tool for neurocritical care specialist. Looking forward, the challenge will be to address the validity of it in different spectrum of brain injuries and to demonstrate that effective treatment can be as a result of it. To advance this field, multinational and multi-institution collaborations will be needed.

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Conflicts of interest

There are no conflicts of interest.

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